

Attachment A

Marked Up Version of Amended Paragraphs

Figure 1 is a graphical representation of the relationship between a probability matrix and a constraint vector of this invention. After a probability matrix is generated, a constraint vector can be applied to the matrix to determine which amino acid substitutions will be selected to test for their effect on a desired functionality. An artificial subtilisin sequence [SEQ ID NO: 2] is depicted as being used to generate a probability matrix, which then is subjected to the application of a constraint vector. In this graphical representation, the residues for which values calculated by the matrix rise above the constraint put on by the vector are candidates for the library.

Figure 2 is an alignment of the sequence of ampC proteins from seven different organisms: organisms (*Aeromonas sobria* [SEQ ID NO: 3], *Enterobacter cloacae* [SEQ ID NO: 4], *Escherichia coli* [SEQ ID NO: 5], *Ochrobactrum anthropi* [SEQ ID NO: 6], *Pseudomonas aeruginosa* [SEQ ID NO: 7], *Salmonella enteritidis* [SEQ ID NO: 8] and *Yersinia enterocolitica* [SEQ ID NO: 9]). A consensus sequence derived as described in Example 2 is also depicted [SEQ ID NO: 10].

A published multiple sequence alignment of 124 subtilisin-like serine proteases (Siezen, et al., *Protein Science* 6:501 (1997)) was recreated from a publicly available database (GENBANK), with the sequence labeled baalkp in the database being substituted with that of GG36. GG36 differs from baalkp by only one residue substitution. In baalkp, residue 87 is an asparagine while in GG36 a serine residue is found at the corresponding position. The GG36 amino acid sequence [SEQ ID NO: 1] was used as the reference sequence, and those positions of the alignment for which the GG36 sequence had a gap character were deleted.

To create the IRL produced in this example, 7 beta lactamase ampC protein sequences (those from *A. sobria*, *E. coli*, *O. anthropi*, *P. aeruginosa*, *sobria* [SEQ ID NO: 3], *E. coli* [SEQ ID NO: 5], *O. anthropi* [SEQ ID NO: 6], *P. aeruginosa* [SEQ ID NO: 7], *S. enteritidis* [SEQ ID NO: 8] and *Y. enterocolitica* [SEQ ID NO: 9]) were aligned using the default parameters of the program AlignX (a component of Vector NTI Suite 6.0 from Informax, Inc.), which is an implementation of the ClustalW alignment algorithm [Thompson, J. D., D. G. Higgins, et al. (1994). *Nucleic Acids Res* 22(22): 4673-80.]. See Figure 2. The sections of the alignment for which the reference sequence (*E. cloacae*; SEQ ID NO: 4) had a gap character were discarded, as only positions at which the reference sequence contained an amino acid were used.